Taxonomy of dark- and white-barked birches related to *Betula pendula* Roth and *B. pubescens* Ehrh. (Betulaceae) in Ukraine based on both morphological traits and DNA markers

ANDRII TARIEIEV[1], IGOR OLSHANSKYI[2], OLIVER GAILING[1], KONSTANTIN V. KRUTOVSKY[1,3,4,5,*]

[1]*Department of Forest Genetics and Forest Tree Breeding, Georg-August University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany*

[2]*M. G. Kholodny Institute of Botany, NAS of Ukraine, Tereshchenkivska St. 2, 01004 Kyiv, Ukraine*

[3]*Laboratory of Population Genetics, N. I. Vavilov Institute of General Genetics, Russian Academy of Sciences, Gubkina Str. 3, Moscow 119991, Russia*

[4]*Laboratory of Forest Genomics, Genome Research and Education Center, Institute of Fundamental Biology and Biotechnology, Siberian Federal University, Akademgorodok 50a/2, 660036 Krasnoyarsk, Russia*

[5]*Department of Ecosystem Science and Management, Texas A&M University, 2138 TAMU, College Station, TX 77843-2138, USA*

*Correspondence author: konstantin.krutovsky@forst.uni-goettingen.de

Running Title: Taxonomy of dark- and white-barked birch

Abstract

Distribution, taxonomy, nomenclature and molecular genetic data are presented for two closely related pairs of birch taxa: 1) dark-barked *Betula pubescens* var. *sibakademica* (Baran.) Kuzeneva (basionym *B. sibakademica* Baranov) and white-barked *B. pubescens* Ehrh. var. *pubescens*, and 2) dark-barked *B. kotulae* Zaverucha = *B. pendula* var. *obscura* (Kotula ex Fiek) Olšavská (basionym *B. obscura* A. Kotula ex Fiek, heterotypic synonym *B. kotulae* Zaverucha) and white-barked *B. pendula* Roth, respectively. Using published data and sequences obtained in this study, it was found that while these pairs can be distinguished not only by morphological characters but also by two diagnostic single nucleotide polymorphisms (SNPs) in the internal transcribed spacers ITS1 and ITS2, no sequence differences within each pair were detected. These results suggest that the color of the bark cannot be used as a taxonomic trait at the species level, and the dark-barked birches within each pair should not be treated as separate species, but as varieties, respectively. Therefore, we reassessed the taxonomical status of *B. sibakademica* and *B. kotulae* using both morphological traits and molecular genetic markers and suggest that *B. pubescens* var. *sibakademica* (Baranov) Kuzeneva and *B. pendula* var. *obscura* (Kotula ex Fiek) Olšavská should be used to name them, respectively. *B. pubescens* var. *sibacademica* was described in Ukraine for the first time.

Keywords: *Betula*, birch, dark-barked, ITS, SNPs, white-barked, taxonomy

INTRODUCTION

Betula pendula Roth and *B. pubescens* Ehrh. are the most common birch species in the flora of Europe and play a very important role in various ecosystems. These species were first described in the 18th century in Germany (Roth, 1788; Ehrhardt, 1791). They are highly polymorphic, include a high number of intraspecific taxa and are known for frequent interspecific hybridization. Some trees in these two species have a dark bark due to the lack of the white pigment betulin. They were described by some researchers as separate taxa, such as *B. obscura* A. Kotula ex Fiek in Poland (Fiek, 1888) or *B. atrata* Domin in the Czech Republic (Domin, 1927). In 1964 Borys Zaverukha described *B. kotulae* Zaverucha as a dark-barked species related to *B. pendula* and in addition proposed to treat *B. obscura* also as another dark-barked species, closely related to *B. pubescens* (Zaverukha, 1964). Similarly, the dark-barked variety *B. pubescens* var. *sibacademica* (Baranov) Kuzeneva was described in Siberia as a separate species *B. sibakademica* Baranov (Baranov, 1924). However, there are still no strong data to support these taxa as separate species considering also the lack of pure populations consisting only of dark-barked trees. The status of these taxa remains unresolved, although some researchers consider them as subspecies or varieties. Meanwhile, these birches were never studied using molecular genetic markers. Thus, we sequenced the ribosomal internal transcribed spacer (ITS) regions or the entire ITS1-5.8S-ITS2 region, which is widely used in molecular phylogeny (Mai and Coleman 1997; Coleman 2000, 2003, 2007, 2009; Wolf et al., 2013) and recently was applied to a number of other birch taxa (Wang et al., 2016).

MATERIALS AND METHODS

Morphological analyses were performed for more than 120 birch specimens of *B. kotulae* Zaverucha (=*B. pendula* Roth var. *obscura* (Kotula ex Fiek) Olšavská) from different herbaria: National Herbarium of Ukraine, M. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kyiv (KW); Herbarium of Kharkiv Botanical Garden, V. N. Karazin

Kharkiv National University, Kharkiv (CWB); Herbarium of V. N. Karazin National Kharkiv University, Kharkiv (CWU), Herbarium of M. M. Gryshko National Botanical Garden, Kyiv (KWHA); Herbarium of O.V. Fomin Botanical Garden, Taras Shevchenko National University of Kyiv, Kyiv (KWHU); Herbarium of Ivan Franko National University of Lviv, Lviv (LW); Herbarium of Institute of Ecology of the Carpathians, National Academy of Sciences of Ukraine, Lviv (LWKS), and Herbarium of State Museum of Natural History, National Academy of Sciences of Ukraine, Lviv (LWS) (Table 1). We also used both literature data (Baranov, 1924; Smyk, 1964; Zaverukha et al., 1986; Didukh et al., 2004) and samples of dark-barked birches collected in our own field trips in Ukraine (Tarieiev and Heluta, 2015) for the description of morphotypes. All the specimens were analyzed by checking the following morphological traits: presence or absence of indumentum on young twigs and leaf plates, shape of the leaf blade, bark color (if sampled from old branches or stem). Based on the data retrieved from herbarium specimens distribution maps were constructed using SimpleMappr (Shorthouse, 2010).

The ITS regions were sequenced and genotyped in 32 herbarium specimens from several herbaria (KW, LE, LW, LWKS, LWS) including both *B. kotulae* and *B. pubescens* var. *sibacademica* (Table 1). DNA was isolated using the Qiagen DNeasy Plant Mini Kit according to the manufacturer's instructions with additional adding of 50 µL 26% polyvinylpyrrolidone **(**PVP) during the procedure. The ITS regions were amplified using two standard PCR primers ITS3 and ITS4 (White et al., 1990) and two modified primers– ITS5 (5'-GGAAGGAAAAGTCGTAACAAGG-3') and ITS2 (5'-GCTACGTTCTTCATCGATGC-3').

Sequencing was performed on an ABI 3130x Genetic Analyser (Applied Biosystems, Thermo Fisher Scientific Inc., Waltham, MA, USA). Quality check, sequence alignment, and sequencing error correction were done using BioEdit (Hall, 1999) and CodonCode Aligner (CodonCode Corporation, Centerville, MA, USA) software.

89 **Table 1** The birch specimens studied using both morphological traits and ITS markers

Name	Storing facility	Specimen ID	ITS markers*	Accession number
Betula kotulae Zaverucha (*B. pendula* Roth var. *obscura* (Kotula ex Fiek) Olšavská)	KW	KW006426 (holotype)	ITS1, ITS2	MH231207 MH042917
		KW008349	ITS	MH178103 MH042912
		KW06427	ITS	MH178104
		KW128013	ITS	MH178105 MH042913
		KW128014	ITS	MH178106
		KW128015	**	
		KW128016	ITS	MH178107
		KW128017	**	
		KW128018	ITS	MH178108
		KW128019	ITS1	MH231208
		KW128020	ITS1	MH231209
		KW128021	ITS	
		KW128022	ITS	MH178109
		KW128023	ITS1, ITS2	MH231210 MH300135
	KWHU	031472	ITS1	MH238474
	LW	LW032757	ITS	
		LW032758	ITS1	MH238475
		LW032759	**	
		LW006898	ITS1, ITS2	MH231213 MH042916
	LWS	LWS27022	ITS1	MH238478
		LWS27026	ITS1	MH238477
	PF	live specimen	ITS1, ITS2	MH231214 MH042918
B. pubescens var. *sibacademica* (Baranov) Kuzeneva	LE	LE01041130 (two different samples on one sheet)	ITS	MH178101 (R) MH231206 (L)
		LE01041129 type specimen,	**	
	KW	KW128012	ITS1, ITS2	MH238471 MH014819
		KW128024	ITS	MH178102 MH042911
	KWHU	031473	ITS1	MH238472, MH238473
	LWKS	LWKS031322	ITS1, ITS2	MH231212 MH042915
	LWS	LWS26648	**	
		LWS26647	**	
B. atrata Domin	KWHU	031470	ITS1, ITS2	MH231211 MH042914

90 *ITS - the complete ITS1-5.8S-ITS2 region was obtained, ITS1 and/or ITS2 – only the spacers
91 were sequenced; **only morphological analysis was done. KW - National Herbarium of
92 Ukraine, M. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kyiv,
93 Ukraine; KWHU - Herbarium of O. V. Fomin Botanical Garden, Taras Shevchenko National
94 University, Kyiv; LW - Herbarium of Ivan Franko National University, Lviv; LWS - Herbarium
95 of State Museum of Natural History, National Academy of Sciences of Ukraine, Lviv; LWKS -
96 Herbarium of Institute of Ecology of the Carpathians, National Academy of Sciences of Ukraine,
97 Lviv; LE - Komarov Botanical Institute, Russian Academy of Sciences, Saint Petersburg,
98 Russian Federation; PF - Potashnya Forestry, Kyiv Region

99

100 Secondary structure reconstruction (Mai and Coleman 1997; Coleman 2000, 2003, 2007,

101 2009; Wolf et al., 2013) was done using MFold (Zucker, 2003) and RNAFold

(http://rna.tbi.univie.ac.at) servers. The correctness of the reconstruction was checked manually by inspecting 5.8S-28S rRNA hybrid stem formation, U-U mismatch in the second loop of ITS2, presence and correct position of conservative motives, and a few other critical aspects. Alignment with secondary structure was done in 4SALE (Seibel 2006, 2008). Visualization was performed in Pseudoviewer (Byun & Han, 2009).

RESULTS

MORPHOLOGICAL ANALYSIS

It was found that all samples could be clearly separated into two groups. One of them consisted of plants with downy young twigs, rounded leaves and morphologically very close to downy birch (*B. pubescens*), except for the dark color of their bark on old branches. The other group consists of plants without indumentum on twigs and leaves, the form of leaf plates is rhombic or rhombic-ovate. This group closely resembled silver birch (*B. pendula*). The pictures of authentic specimens are presented for comparison in Figures 1 and 2.

<Figures 1 & 2>

DISTRIBUTION

White-barked *Betula pubescens* Ehrh. var. *pubescens* is distributed in Polissia (woodlands) and Lisostep (forest-steppe) zones (Fig. 3). However, its morphological identification is difficult because this species is polymorphic and has a high morphological plasticity. There are also cases of interspecific hybridization between *B. pubescens* and *B. pendula* Roth in northern and central regions of Ukraine. Morphological evidence for hybridization of both *B. pubescens* and *B. pendula* with a local endemic species *B. borysthenica* Klokov can be observed in the southern part of their natural habitat (in particular in Cherkasy, Dnipro (former name – Dnipropetrovsk) and Zaporizhzhia Regions), where the distribution range of *B. pubescens* and *B. pendula* partly overlaps with the range of *B. borysthenica* Klokov that grows mainly in the Kherson Region (Oleshkivski Sands) (unpublished authors' observation).

<Figure 3>

Dark-barked *B. pubescens* Ehrh. var. *sibakademica* (*B. pubescens* Ehrh. var. *sibakademica* (Baranov) Kuzeneva, 1936, Fl. USSR, 5: 296. ≡ *B. sibakademica* Baranov, 1924, Izv. Zapadno-Sibirsk. Otd. Russk. Geogr. Obshch. 4(1): 52) differs from the white-barked variety *B. pubescens* var. *pubescens* by the brownish black color of the bark on its stem due to the lack of the white pigment betulin. This variety was first described in Omsk, southwestern Siberia, the Russian Federation (Baranov, 1924). Authentic materials are kept in LE, but a lectotype was not selected.

In Ukraine *B. pubescens* var. *sibakademica* occurs in both Polissia and Lisostep zones (Fig. 4). This variety is known in a few locations only: Lviv, E. Wołoszczak, VII. 1892, LW 032680; Lviv Region, Yavoriv District, outskirts of Ivano-Frankove (Yaniv), urban settlement, K. Malynovskyi, 27. V. 1949, LWS 26647! and LWS 26648!; Volyn Region, Shatsk District, outskirts of Svitiaz vill., 22.VI. 1982, V. Kudryk & V. Terletskyi, KW!; Rivne Region, Radyvyliv District, outskirts of Krupets Village, O. Kagalo, V. Batochenko, A. Tareiev & I. Bednarska, 10. VIII. 2014, LWKS 031322!; Zhytomyr Region, Ovruch District, outskirts of Chervonka Village, Osnyie natural landmark (Smyk, 1964); Zhytomyr Region, Ovruch District, outskirts of Mamech Village (Zaverucha et al., 1986); Zhytomyr Region, outskirts of Yemilchyne urban settlement (Zaverucha et al., 1986); Chernihiv Region, Kozelets District, Nadynivka Village, 07.VII. 1999, V. Melnyk, KWHA!.

<Figure 4>

Dark-barked downy birch trees occur in the southern part of the range (distribution) of *B. pubescens* (Fig. 3). Outside Ukraine *B. pubescens* var. *sibakademica* is known in Poland (Jentyś-Szaferova, 1959; Skvortsov, 1986), Kazakhstan (Petrov, 1964; Naumenko, 2012), the Russian Federation (Baranov, 1924; Skvortsov, 1986; Naumenko, 2008, 2012), and probably in Latvia (Skvortsov, 1986). We suppose that it can be found in other countries as well.

White-barked *Betula pendula* Roth var. *pendula* is distributed across the entire Ukraine with major occurrences in Polissia (woodlands) and Lisostep (forest-steppe) zones. However, this species also occurs in more southern regions and even across the southern shore of Crimea. The distribution map based on herbarium specimens is presented in Fig. 5.

<Figures 5>

Dark-barked *Betula pendula* Roth var. *obscura* (Kotula ex Fiek) Olšavská (*B. pendula* Roth var. *obscura* (Kotula ex Fiek) Olšavská, 2006, Fl. Slovenska 5(3): 149. ≡ *B. obscura* Kotula ex Fiek, 1888, Jahresber. Schles. Ges. Vaterl. Cult. 65: 314 (-315). = *B. kotulae* Zaver., 1964, Ukr. Bot. J. 21(5): 83) differs from the white-barked variety of silver birch *B. pendula* Roth by the dark brownish color of their bark. The distribution of this variety (Fig. 6) asymmetrically overlaps with the range of the white-barked variety of silver birch (Fig. 5). All findings of the dark-barked variety were in the northwestern Ukraine.

<Figures 6>

INTERNAL TRANSCRIBED SPACER NUCLEOTIDE MARKERS

Internal transcribed spacers ITS1 and ITS2 were sequenced in 21 herbarium specimens and one living sample (Table 1) which represented five samples of *B. pubescens* var. *sibakademica*. and 17 of *B. kotulae*. In addition, we analyzed publicly available sequences of the same region for *B. pendula* (NCBI GenBank accession numbers AM503889.2, KT308990.1-KT308991.1, KT308996.1-KT309008.1, JN247411.1, FJ011777.1, AY761127.1, and AY352332.1) and *B. pubescens* (KT308969.1-KT308976.1, KT308980.1-KT308983.1, and AY761130.1), which were retrieved from the NCBI GenBank. There are no sequencing data available for the authentic reference specimens (type specimens) representing *B. pendula* and *B. pubescens.*

We found two single nucleotide polymorphisms (SNPs) in the ITS1 and ITS2 regions, respectively, that had different alleles between the two groups of species, *B. pubescens* vs. *B. pendula*, but the same alleles within each group.

One of these polymorphisms, G/T SNP, was located in the ITS1 locus and corresponds to G or U in the first RNA loop (H1) in the ITS1 structure, respectively. It can cause a structural change there. The G→T nucleotide substitution in the ITS1 locus causes T→U nucleotide substitution in the first RNA loop (H1) that creates a mismatching U-U pair (Fig. 7). Both dark- and white-barked birches within the *B. pubescens* group had allele G, but both dark- and white-barked birches within the *B. pendula* group had another allele T.

Another SNP (C/T) was located in the ITS2 locus and corresponds to C or U in the fourth RNA helix (H4) in the ITS2 structure. Similar to the first SNP, both dark- and white-barked birches within the *B. pubescens* group had the same allele C, but both dark- and white-barked birches within the *B. pendula* group had another allele T (Fig. 8).

<Figures 7 & 8>

DISCUSSION

Two SNPs found in this study can be used as diagnostic sites to discriminate the two groups of species, *B. pubescens* vs. *B. pendula*. Moreover, the C→U nucleotide substitution in H4 in the ITS2 structure represents a hemicompensatory base change (hCBC) when a conventional base pair G-C is substituted by a wobble one G-U (Fig. 8). Changes such as hCBC and CBC were proposed as markers to discriminate different taxa (Coleman, 2009; Wolf, 2013). Both SNPs G/T(U) and C/T(U) in the ITS1 and ITS2 loci, respectively, allowed us to discriminate the two groups of species, but the later one not as clearly as the former one, because two sequences in GenBank (KT308969.1 and KT308970) listed as downy birch (*B. pubescens*) have allele T(U) in H4 (ITS2) instead of the expected C, but allele C in H1 (ITS1) as expected. However, recombinant ITS, morphological misidentification due to frequent interspecific hybridization between *B. pendula* and *B. pubescens* or misgenotyping could explain this sequence discrepancy.

CONCLUSIONS

As far as we know this is the first study describing *B. pubescens* var. *sibacademica* in Ukraine. Taxa identification was based on a number of herbarium specimens including authentic material and done using both traditional morphological and molecular genetic analyses.

The taxonomical status of dark-barked birches *B. pendula* var. *obscura* and *B. pubescens* var. *sibacademica* and their distribution across Ukraine were reassessed based on genotyping herbarium collections using molecular genetic markers. Obtained results assured us that dark-barked birches should not be considered as separate species, but as varieties of *B. pendula* and *B. pubescens*, respectively. However, to further confirm this conclusion additional studies need to be conducted based on additional molecular genetic markers, chromosome number counting, etc. It would be also interesting to study genes involved in the betulin synthesis (Yin et al., 2011) in both dark- and white-barked varieties of both *B. pendula* and *B. pubescens*.

ACKNOWLEDGEMENTS

Authors are grateful to Dr. Natalia M. Shiyan, curator of National Herbarium of Ukraine (KW); Prof. Dr. Sergei L. Mosyakin, Director of M. G. Kholodny Institute of Botany; Prof. Dr. Dmitry V. Geltman, Director of BIN RAS; Dr. Maryna V. Legchenko, BIN RAS; Dr. Tetiana S. Khmil, curator of Herbarium of Ivan Franko National University of Lviv (LW); Dr. Nadiya M. Sytschak, curator of Herbarium of Institute of Ecology of the Carpathians (LWKS); Kateryna M. Danyliuk, curator of Herbarium of State Museum of Natural History(LWS); Mr. Andrii I. Girin, O.V. Fomin Botanical Garden for the samples and to Oleksandra Dolynska for technical help with DNA isolation and sequencing. We thank the German Academic Exchange Service (DAAD, Deutsche Akademischer Austauschdienst) for providing a short-term research scholarship and a PhD fellowship to Andrii Tarieiev for this research.

REFERENCES

Ashburner K, McAllister HA. 2013. The genus *Betula*: a taxonomic revision of birches. Kew: Kew Publishing.

Baranov VI. 1924. Notes about birches from Western Siberia. *Reports of Western-Siberian Department of Russian Geographical Society* **4(1):** 47–54. In Russian [**Баранов ВИ. 1924** Заметки о березах из лесостепи Западной Сибири. *Известия Западно-Сибирского Отдела Русского географического общества* **4(1):** 47–54.]

Brown IR, Kennedy D, Williams DA. 1982. The occurrence of natural hybrids between *Betula pendula* Roth and *B. pubescens* Ehrh. *Watsonia* **14:** 133–145.

Byun Y and Han K. 2009. PseudoViewer3: generating planar drawings of large-scale RNA structures with pseudoknots, *Bioinformatics* **25:** 1435-1437.

Coleman AW. 2000. The significance of a coincidence between evolutionary landmarks found in mating affinity and a DNA sequence. *Protist* **151:** 1–9.

Coleman AW. 2003. ITS2 is a double-edged tool for eukaryote evolutionary comparisons. *Trends in Genetics* **19:** 370–375.

Coleman AW. 2007. Pan-eukaryote ITS2 homologies revealed by RNA secondary structure. *Nucleic Acids Research* **35:** 3322–3329.

Coleman AW. 2009. Is there a molecular key to the level of "biological species" in eukaryotes? A DNA guide. *Molecular Phylogenetics and Evolution* **50:** 197–203.

De Jong PC. 1993. An introduction to *Betula*: its morphology, evolution, classification and distribution, with a survey of recent work. In: Hunt DR., ed. *Proceedings of the IDS Betula symposium, 2–4 October 1992*. Richmond: International Dendrology Society, 7–18.

Didukh YaP, Fitsaylo TV, Burda RI et al. 2004. *Betula pubescens* Ehrh. In: Didukh YaP ed. *Ecoflora of Ukraine.* Kyiv: Phytosociocentre, **2:** 432–433. In Ukranian [**Дідух ЯП, Фіцайло ТВ, Бурда РІ та ін. 2004.** *Betula pubescens* Ehrh. В: Дідух ЯП ред. *Екофлора України*. Київ: Фітосоціоцентр, **2:** 432–433.]

Domin K. 1927. *Betula atrata* Domin, nová břiza z Českomoravské vysočiny. *Rozpravy II třídy České akademie* **36(52):** 1–10. In Czech.

Ehrhart F. 1791. Beiträge zur Naturkunde, und den damit verwandten Wissenschaften, besonders der Botanik, Chemie, Haus- und Landwirthschaft, Arzneigelahrtheit und Apothekerkunst. Hannover & Osnabrück: Verlage bei Christian Ritscher **6:** 184.

Fiek E. 1888. Resultate der Durchforschung der schlesischen Phanerogamenflora im Jahre 1887. *Jahres-Bericht der Schlesischen Gesellschaft für Vaterländische Cultur* **65:** 309–349.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41:** 95-98.

Jentys-Szaferowa J. 1959. Problematyka brzozy czarnej (*Betula obscura* Kotula). *Rocznik Sekcji dendrologicznej Polskiego towarzystwa botanicznego* **13:** 11-66. In Polish.

Johnsson H. 1945. Interspecific hybridization within the genus *Betula*. *Hereditas* **31:** 163–176.

Johnsson H. 1949. Studies on birch hybrids. *Hereditas* **35(2):** 115–135.

Kagalo OO, Skybitska NV. 2009. *Betula obscura* A.Kotula (incl. *B. kotulae* Zaverucha; *B. pendula* Roth subsp. *obscura* (A.Kotula) Á.Löve, *B. verrucosa* Ehrh. subsp. *obscura* (A.Kotula) Á.Löve et D.Löve) In: Didukh YaP, ed. *Red Book of Ukraine. Plants*. Kyiv: Globalconsulting. In Ukranian [**Кагало ОО, Скібіцька НВ. 2009.** Береза темна *Betula obscura* A.Kotula (incl. *B. kotulae* Zaverucha; *B. pendula* Roth subsp. *obscura* (A.Kotula) Á.Löve, *B. verrucosa* Ehrh. subsp. *obscura* (A.Kotula) Á.Löve et D.Löve) В: Дідух ЯП, ред. *Червона книга України. Рослинний світ.* Київ: Глобалконсалтинг.]

Koetschan C, Hackl T, Müller T, Wolf M, Förster F, et al. 2012. ITS2 database IV: interactive taxon sampling for internal transcribed spacer 2 based phylogenies. *Molecular Phylogenetics and Evolution* **63:** 585–588.

Kuzeneva, IO. 1936. Betuleae Döll. In: Komarov VL, ed. *Flora of the USSR* **5:** 296. [**Кузенева ИО. 1936.** Betuleae Döll. В: Комаров ВЛ, ред. *Флора СССР* **5:** 296. In Russian]

Linnaeus C. 1753. Species Plantarum. Holmiae: Impensis Laurentii Salvii. **2:** 1200 pp.

Mai JC, Coleman AW. 1997. The internal transcribed spacer 2 exhibits a common secondary structure in green algae and flowering plants. *Journal of Molecular Evolution* **44:** 258–271.

Mosyakin SL, Fedoronchuk MM. 1999. Vascular plants of Ukraine: A nomenclatural checklist. Kiev.

Naumenko NI. 2008. Flora and vegetation of Southern Ural. Kurgan: Publishing house of Kurgan state university. In Russian [**Науменко НИ. 2008.** Флора и растительность Южного Зауралья. Курган: Изд-во Курганского гос. ун-та.]

Naumenko NI. 2012. *Betula pubescens* Ehrh. var. *sibacademica* Baranov. In: Red Book of the Kurgan Region. Kurgan: Publishing house of Kurgan state university. In Russian [**Науменко НИ. 2012.** *Betula pubescens* Ehrh. var. *sibacademica* Baranov. В кн.: Красная книга Курганской области. Курган: Изд-во Курганского гос. ун-та.]

Olšavská, K. 2006. Breza previsnutá tmavá *Betula pendula* var. *obscura* (Kotula ex Fiek) Olsavská, comb, nova. In: Goliašová K, Michalková E., eds. *Flóra Slovenska* **5(3):** 149. In Slovenian.

Olshanskyi IG, Shyian NM, Tarieiev AS. 2016. Types of *Betula* (Betulaceae) names described from Ukraine, kept in the National Herbarium of Ukraine (KW). *Ukrainian Botanical Journal* **73(4):** 401–403.

Roth AW. 1788. Tentamen florae Germanicae; continens enumerationem plantarum in Germania sponte. Lipsiae: in Bibliopolio I.G. Milleriano **1:** 405

Seibel PN, Müller T, Dandekar T, Schultz J, Wolf M. 2006. 4SALE - A tool for synchronous RNA sequence and secondary structure alignment and editing. *BMC Bioinformatics* **7:** 498.

Seibel PN, Müller T, Dandekar T, Wolf M. 2008. Synchronous visual analysis and editing of RNA sequence and secondary structure alignments using 4SALE. *BMC Research Notes* **1:** 91.

Tarieiev AS, Heluta VP. 2015. A new record of *Betula obscura* (Betulaceae) from Central Polissya of Ukraine. *Ukrainian Botanical Journal* **72(2):** 144-146. In Ukranian with English Abstract.

Thórsson ÆTh., Salmela E, Anamthawat-Jónsson K. 2001. Morphological, cytological, and molecular evidence for introgressive hybridisation in birch. *Journal of Heredity* **92(5):** 404–408.

Wang N, McAllister HA, Bartlett PR, Buggs RG. 2016. Molecular phylogeny and genome size evolution of the genus *Betula* (Betulaceae). *Annals of Botany* **117(6):** 1023-1035.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR Protocols: a guide to methods and applications.* Academic Press, New York, USA.

Wolf M, Chen S, Song J, Ankenbrand M, Müller T. 2013. Compensatory base changes in ITS2 secondary structures correlate with the biological species concept despite intragenomic variability in ITS2 sequences - A Proof of Concept. *PLoS ONE* **8(6):** e66726.

Yin, J., Ren, CL., Zhan, YG. et al. 2012. Distribution and expression characteristics of triterpenoids and OSC genes in white birch (*Betula platyphylla* Suk.). *Molecular Biology Reports* **39:** 2321

Zaverucha BV. 1964. New and rare birch species of Ukrainian flora. *Ukrainian Botanical Journal* **21(5):** 78-86. [**Заверуха БВ. 1964.** Нові та рідкісні види берез української флори. *Український ботанічний журнал* **21(5):** 78-86. In Ukranian with English summary.]

Zaverukha BV, Ivchenko IS, Kozyakov OS. 1986. Dark-barked birches of Ukraine. *Ukrainian Botanical Journal* **43(3):** 79–83. [**Заверуха БВ, Івченко ІС, Коз'яков ОС. 1986.** Темнокорі берези України. *Український ботанічний журнал* **43(3):** 79–83. In Ukranian with English summary.]

Zuker M. 2003. MFold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research* **31(13):** 3406-3415.

Figure Legends

Figure 1. *Betula pubescens* var. *sibacademica* authentic specimen collected by V. Baranov (LE01041130).

Figure 2. *Betula kotulae* holotypus authentic specimen collected by B. Zaverukha (KW006426).

Figure 3. Distribution of *Betula pubescens* Ehrh. var. *pubescens* in Ukraine based on herbarium specimens.

Figures 4. Distribution of *Betula pubescens* Ehrh. var. *sibakademica* (Baranov) Kuzeneva in Ukraine based on herbarium specimens and literature data.

Figures 5. Distribution of *Betula pendula* Roth in Ukraine based on herbarium specimens.

Figure 6. Distribution of *Betula pendula* Roth var. *obscura* (Kotula ex Fiek) Olšavská in Ukraine based on herbarium specimens.

Figure 7. The ITS1 secondary structure and differences in the H1 helix. The G/T(U) SNP in *B. pubescens* var. *sibacademica* and *B. pubescens* var. *pubescens* (allele G); and in *B. pendula* var. *obscura* and *B. pendula* var. *pendula* (allele T(U)).

Figure 8. The ITS2 secondary structure and differences in the H4 helix. The C/T(U) SNP in *B. pubescens* var. *sibacademica* and *B. pubescens* var. *pubescens* (allele C), and in *B. pendula* var. *obscura* and. *B. pendula* var. *pendula* (allele T(U)).